

## Effect of Cyclanilide, Ethephon, Auxin Transport Inhibitors, and Temperature on Whole Plant Defoliation

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### ABSTRACT

Whole plant defoliation studies were performed using dark red kidney bean (*Phaseolus vulgaris* L.) to compare the effect of cyclanilide [1-(2,4-dichlorophenylaminocarbonyl)-cyclopropane carboxylic acid], ethephon (2-chloroethylphosphonic acid), auxin transport inhibitors, and temperature on leaf abscission. Ethephon induced bean leaf defoliation, but ethephon combined with cyclanilide induced greater defoliation than ethephon alone. The response was dose dependent, as 0.067 kg/ha ethephon alone did not induce defoliation, but when combined with an equal rate of cyclanilide induced 63% defoliation five days after treatment (DAT). In addition, combining auxin transport inhibitors at 0.140 kg a.i./ha with 0.067 kg a.i./ha ethephon induced 50 to 52% defoliation, 5 DAT. Ethephon-induced defoliation was more temperature sensitive than the combination of cyclanilide with ethephon. Ethephon alone (0.067 kg a.i./ha) induced 26% defoliation 5 DAT at the highest tested temperature (30/26°C, day/night), but cyclanilide combined with ethephon induced 75 to 85% defoliation at all tested temperatures (except at 16/14°C, the lowest). These results indicate that cyclanilide is an ethephon synergist because it enhanced ethephon activity, even at low temperatures. These results support the model that ethylene released by ethephon induces abscission, and suggests that inhibition of auxin transport or auxin signaling will increase the effect of ethylene on the process of leaf abscission. Because of the similarities to the activity to 1-N-naphthylphthalamic acid (NPA) and 2,3,4-triiodobenzoic acid (TIBA) in vivo, cyclanilide may act as an auxin transport inhibitor.

**D**EFOLIANTS AND DESICCANTS are classified as harvest aid chemicals because they are commonly used to facilitate mechanical harvesting of crops. In cotton (*Gossypium hirsutum* L.), defoliation prior to harvest is important because the pigments in the leaves can stain and reduce the quality of the cotton fiber (Nickell, 1982, p. 19–27). In addition, removal of foliage prior to harvest will result in cotton that has less debris. Defoliants or desiccants are also used to synchronize and enhance boll opening in cotton, thus allowing the farmer to obtain maximum yield in a single harvest. In addition to cotton, soybean [*Glycine max* (L.) Merr.], rice (*Oryza sativa* L.), potato (*Solanum tuberosum* L.), grain sorghum [*Sorghum bicolor* (L.) Moench], sunflower (*Helianthus annuus* L.), lentil (*Lens culinaris* Medik.), trefoil (*Lotus* spp.), dry bean (*Phaseolus vulgaris* L.), and sugarcane (*Saccharum* spp.) are often desiccated to facilitate harvest (Yang, 1986). In fruit crops such as olive (*Olea europaea* L.), apple (*Malus* spp.), pear (*Pyrus* spp.), peach [*Prunus persica* (L.) Batsch], and citrus (*Citrus* spp.), chemicals

that act as defoliants are used to facilitate the loosening of fruit from trees prior to mechanical harvesting or to synchronize fruit ripening (Cooper et al., 1968; Edgerton and Blanpied, 1968). Defoliants and desiccants are therefore used to increase crop yield, improve crop quality, and improve harvest efficiency.

FINISH is a harvest aid chemical produced by Bayer Crop Science, Monheim, Germany, labeled for use in cotton. It is used to enhance defoliation, accelerate boll opening, and prevent terminal regrowth (FINISH label, Bayer Crop Science). FINISH contains two active ingredients: ethephon and cyclanilide. Ethephon was initially registered for use in 1971, but was not used in cotton until the mid 1980s. In the mid 1990s, FINISH was introduced, because ethephon efficacy in cotton was enhanced when combined with the compound cyclanilide (Stewart et al., 2000). The use of FINISH compares favorably with other cotton harvest aid treatments when evaluated on yield and harvest quality (Larson et al., 2002). Cyclanilide has also been used alone to induce lateral branching in apple trees (Elfving and Visser, 2005). Cyclanilide is a malonanilate, and has a cyclopropane ring similar to 1-aminocyclopropane-1-carboxylic acid (Fig. 1).

Defoliation or leaf abscission is induced in plants as a natural process in response to environmental changes, pest or pathogen attack, or can be induced with the application of synthetic chemicals (Osborne, 1989; Roberts et al., 2002). The plant hormones auxin and ethylene are the main plant growth regulators which control natural leaf abscission. While auxin suppresses abscission, ethylene promotes abscission (Addicott, 1982; Taylor and Whitelaw, 2001). Applying auxin at a distal position in reference to the abscission zone can inhibit leaf abscission even after the abscission process has begun (Chatterjee and Leopold, 1963). Combinations of auxin and ethylene induced the transdifferentiation of cortical cells to abscission cells (McManus et al., 1998). Synthetic auxins such as 1-Naphthaleneacetic acid and 2,4-dichlorophenoxyacetic acid have commonly been used in fruits to prevent yield loss by inhibiting early fruit drop or abscission (Cooper et al., 1968).

Ethylene is the main hormone that promotes abscission by inducing the production of various cell wall hydrolases in the abscission zone to stimulate cell wall breakdown and eventual shedding (Campillo and Lewis, 1992; Sexton and Roberts, 1982). The hormone balance model for leaf abscission proposes that there are two general temporal phases in an expanding leaf (Rubinstein and Leopold, 1963; Osborne, 1989; Taylor and

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**Abbreviations:** DAT, days after treatment; IAA, indole-3-acetic acid; MDR, multidrug resistance; NPA, 1-N-naphthylphthalamic acid; TIBA, 2,3,4-triiodobenzoic acid.

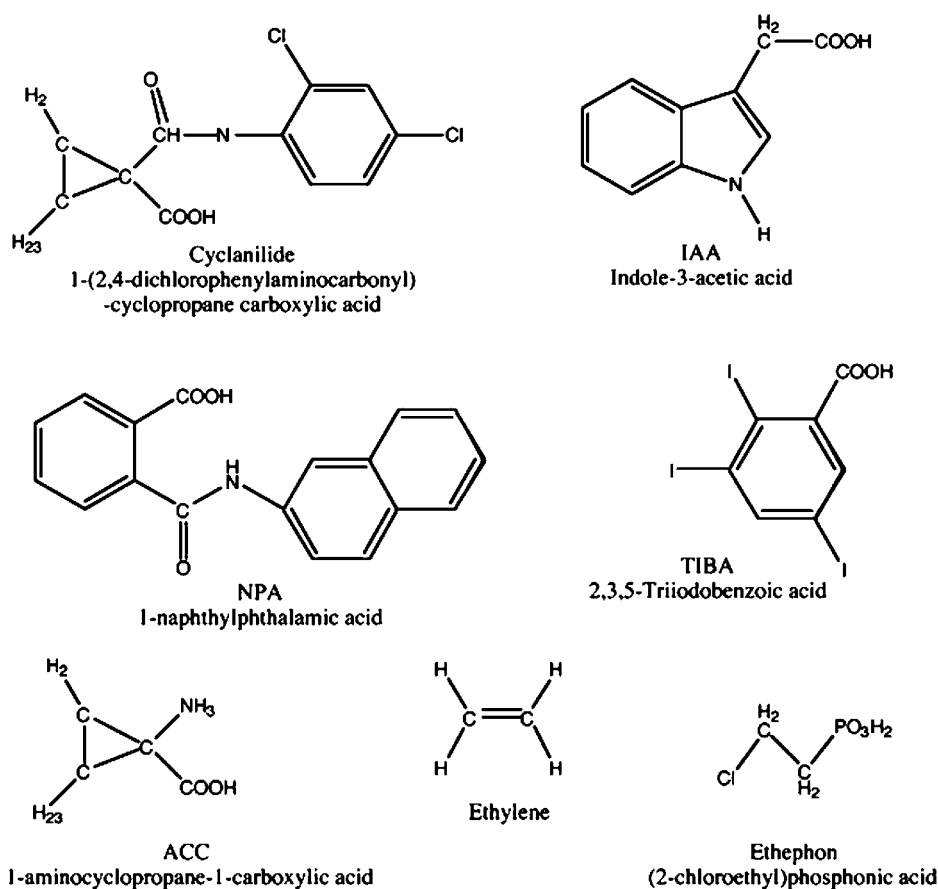


Fig. 1. Structures of chemicals used in the research studying leaf abscission (cyclanilide, NPA, TIBA, and ethephon). Structures of IAA, ethylene, and ACC are included because they are endogenous compounds involved in abscission.

Whitelaw, 2001; Roberts et al., 2002). During growth and maintenance phases of the leaf, auxin suppresses abscission and ensures that the leaf remains viable and attached to the plant. This is followed by the terminal phase that is characterized by a decline in auxin transport through the petiole, and an increase in ethylene levels (Morgan et al., 1992). Eventually, with the degradation of the cell walls in the abscission zone, and the help of gravitational forces, the leaf is shed.

Hormonal defoliant is a type of harvest aid chemicals that mimic the natural process of defoliation or leaf abscission (Morgan, 1985). Ethephon is an example of a chemical that is commonly used in cotton as a preharvest aid treatment to induce defoliation and enhance boll opening (Morgan, 1969). Ethephon is applied to the foliage, and after entering the leaf tissue, is degraded under alkaline conditions producing ethylene, phosphate, and chloride ions (Yang, 1969; Warner and Leopold, 1969). Although ethephon will induce defoliation without injuring the crop, it has some limitations. For example, at low field temperatures (<15.5°C at night, Edmisten, 2005), ethephon efficacy decreases because degradation to ethylene is temperature dependent (Olien and Bukovac, 1978). This can be a problem because cotton is harvested in the late summer or early fall when temperatures begin to decrease. In addition, ethephon does not inhibit regrowth after defoliation. These problems

can be overcome by combining ethephon with an additional chemical, cyclanilide.

Cyclanilide does not induce ethylene production. According to the hormone balance model for leaf abscission (Addicott, 1982; Taylor and Whitelaw, 2001), auxin concentration within, or transport through the petiole must decrease for ethylene to promote defoliation. We therefore hypothesize that cyclanilide may enhance ethephon efficacy by suppressing the auxin signaling system in the petiole, thereby allowing ethylene released by the degradation of ethephon to induce more defoliation.

The overall purpose of our research is to understand the mechanism by which cyclanilide enhances defoliation. Toward this goal, we studied the effect that ethephon, or ethephon plus cyclanilide, had on dark red kidney bean defoliation. We performed these experiments using greenhouse and controlled environmental conditions to determine if temperature had an effect on defoliation. We hypothesize that cyclanilide's mechanism of action may be as an auxin signal or transport inhibitor. We chose to use kidney bean plants for the defoliation experiments because they have often been used in understanding the physiology of leaf abscission (Wright and Osborne, 1974; Addicott, 1982; Campillo and Lewis, 1992; McManus et al. 1998). Ethylene and auxin have been found to regulate the abscission process in all plants studied (Taylor and Whitelaw, 2001). Uti-

lizing an experimental system that would allow us to rapidly and reproducibly evaluate the effect of ethephon and cyclanilide on abscission is needed to obtain the fundamental information about the interaction. This information will then be the basis for experimental work with cotton and other crops in field situations.

## MATERIALS AND METHODS

### Plant Material

Kidney bean seeds were germinated in sand for 1 wk. After emergence, seedlings were transferred to 10.2-cm pots containing Pro-Mix (Premier Horticulture Ltd., Dorval, QC, Canada) soil and watered as needed. Plants were grown in the greenhouse with an average midday light intensity of  $850 \mu\text{mol}/\text{m}^2/\text{s}^2$  and day/night temperatures of  $26/21 \pm 5^\circ\text{C}$ . Plants were sprayed 3 wk after planting when the first, second, and third trifoliates were fully expanded and the fourth trifoliolate had emerged. The average number of leaves per plant at this stage was 14. Prior to spray treatments, the soil in the pots was covered with vermiculite to prevent soil contact of the chemicals. After spray treatments, vermiculite was removed and plants were watered at the base of the plant throughout the remainder of the experiment.

### Chemicals

Spray treatments were prepared in water and consisted of formulated ethephon (Prep 55.4), formulated cyclanilide (WP 50), technical grade TIBA, and NPA. Ethephon and cyclanilide were donated by Rhne Poulenc Agro (Raleigh, NC), and TIBA and NPA were purchased from Sigma Chemical Co. (St. Louis, MO) and Chem Service Inc. (West Chester, PA), respectively. The TIBA and NPA stock solutions were prepared by dissolving the chemicals in acetone and 1 M NaOH, respectively. Stock solutions were then diluted with water to obtain concentrations equal to the rates at which cyclanilide and ethephon were applied so that the final concentration of acetone or NaOH did not exceed 0.1%. The TIBA and NPA solutions were adjusted to pH 2.5 with HCl to equal the pH of formulated ethephon and cyclanilide solutions. Triton at 0.1% was also included in TIBA and NPA spray solutions as a surfactant.

### Spray Treatments

Chemicals (cyclanilide, TIBA, and NPA) were applied at 0.017, 0.034, 0.067, 0.100, 0.140, and 0.561 kg a.i./ha either alone or in combination with ethephon. Control treatment consisted of water alone. Spray treatments were applied using a DeVries (Hollandale, MN) spray chamber calibrated to deliver 383 L/ha at 5 km/h using a Teejet (Wheaton, IL) 8006 nozzle.

### Temperature Studies

Kidney bean plants were grown as described above in controlled environmental chambers at  $26/22^\circ\text{C}$  day/night temperatures. The average photosynthetic photon flux density of the chambers was  $600 \mu\text{mol}/\text{m}^2/\text{s}^2$ . Plants were grown with a 9-h plus a 3-h night interruption photoperiod equal to a long day across 3 wk. Three-week-old kidney bean plants were sprayed with 0.067 kg a.i./ha ethephon alone, or in combination with 0.067 kg a.i./ha cyclanilide. Water was used as a control. After spray treatments, plants were placed in chambers set at the desired temperature with the same long-day photoperiod. Day/night temperatures tested were 30/26, 26/22, 22/18, 18/16, and 16/14°C.

## Data Analysis

After spray treatments, the number of leaves on each plant were counted each day during a 1-wk period. Percentage defoliation was calculated from the number of leaves remaining the day of measurement ( $L_n$ ) divided by the total number of leaves at the time of chemical application ( $L_1$ ) times 100 [i.e.,  $(L_n/L_1) \times 100$ ]. The experiments were set up in a completely randomized design with three replications per treatment, and experiments were repeated. Data was analyzed as a three-way factorial using JMP SAS (Cary, NC) software. The calculated error variance between repeated experiments were in all cases homogenous, thus data were combined for presentation. Factors analyzed were chemical applied, rate of application, and day at which measurements were taken. Data are presented as mean  $\pm$  SE percentage defoliation.

## RESULTS

### Greenhouse Studies

Whole plant experiments in the greenhouse were conducted to compare the effect of ethephon alone or in combination with cyclanilide and auxin transport inhibitors such as TIBA and NPA. Without ethephon, the cyclanilide, NPA, and TIBA treatments did not induce any defoliation. However, compared with the control, plant growth was altered by these chemicals. No differences in the number of leaves between control and treatments were observed during a 1-wk period, but there were differences in the growth pattern between the control and cyclanilide treatments (data not shown). Control plants grew taller and were more elongated, and proceeded with vining characteristics that are typical of bean plants. In contrast, cyclanilide induced a proliferation of lateral shoot growth and inhibited apical growth. These observations are similar to the findings of Elfving and Visser (2005), which demonstrated the application of cyclanilide caused the induction of lateral branching in apple trees. Therefore, cyclanilide alone acts as a plant growth regulator, but with effects that are very different from those of ethylene.

From our whole plant studies we determined that defoliation was dependent on three factors: time, type of chemical, and rate of application (each factor:  $P = 0.001$ ). Combining ethephon with cyclanilide enhanced defoliation compared with ethephon alone (Fig. 2). Ethephon applied at the highest tested rate (0.140 kg a.i./ha) induced 75% defoliation 7 DAT. Ethephon alone at half this rate (0.067 kg a.i./ha) did not induce any defoliation 7 DAT. Combining 0.140 kg a.i./ha cyclanilide with 0.067 kg a.i./ha ethephon induced 100% defoliation by 6 DAT. These results demonstrated that combining cyclanilide with a low rate of ethephon resulted in greater defoliation in less time than the application of a high rate of ethephon alone.

At 5 DAT, increasing rates of ethephon alone induced a maximum of 50% defoliation at the highest rate (Fig. 3). While 0.067 kg a.i./ha ethephon alone did not induce any defoliation 5 DAT, combining this low rate of ethephon with increasing rates of cyclanilide resulted in 63 to 80% defoliation. In fact, cyclanilide applied at 0.017 to 0.034 kg a.i./ha combined with 0.067 kg a.i./ha

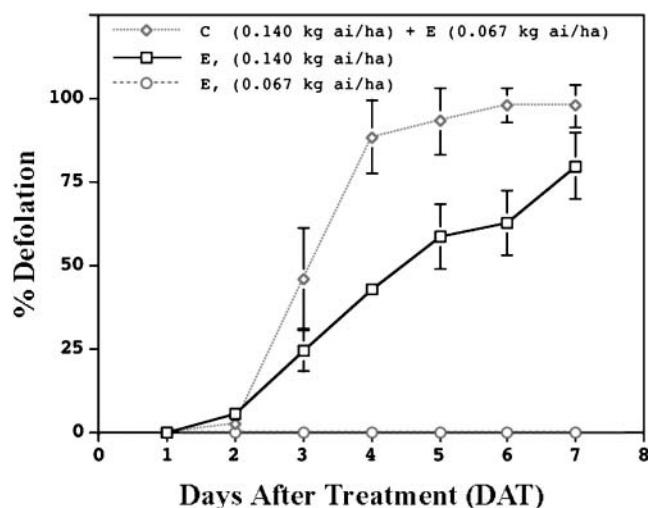


Fig. 2. Defoliation of dark red kidney beans across time with ethephon (E) alone at 0.140 kg a.i./ha and 0.067 kg a.i./ha, and cyclanilide (C) at 0.140 kg a.i./ha combined with ethephon at 0.067 kg a.i./ha. Each point represents mean  $\pm$  SE,  $n = 3$ .

ethephon resulted in 55 to 70% defoliation. These results demonstrated that low rates of cyclanilide combined with inactive rates of ethephon will enhance defoliation.

Low rates of ethephon combined with low rates of cyclanilide did not induce defoliation (Table 1). At least 0.067 kg a.i./ha ethephon was necessary to induce defoliation when combined with lowest rate of cyclanilide. For example, combining 0.067 kg a.i./ha cyclanilide with 0.034 kg a.i./ha ethephon did not induce any defoliation across time, while reversing the rates so that cyclanilide was applied at 0.034 kg a.i./ha and ethephon was applied at 0.067 kg a.i./ha resulted in 55% defoliation 5 DAT. Cyclanilide applied at high rates with low ethephon rates also enhanced defoliation. Combining 0.140 kg a.i./ha cyclanilide with the lowest ethephon rate (0.017 kg a.i./ha) resulted in 23% defoliation 5 DAT.

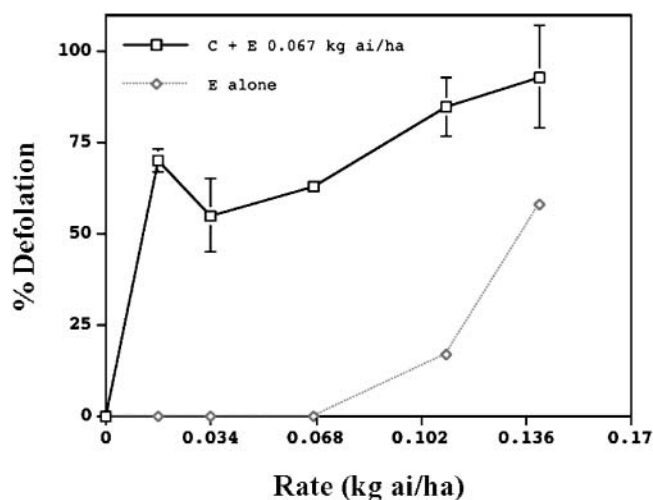


Fig. 3. Defoliation (5 DAT) of dark red kidney beans sprayed with increasing rates of ethephon (E) alone, and increasing rates of cyclanilide (C) combined with 0.067 kg a.i./ha ethephon. Each point represents mean  $\pm$  SE,  $n = 3$ .

Table 1. Percentage defoliation of kidney bean plants, 5 DAT, comparing combinations of different concentrations of ethephon and cyclanilide. Each point represents mean  $\pm$  SE,  $n = 3$ .

Ethephon kg a.i./ha	Cyclanilide, kg a.i./ha					
	0	0.017	0.034	0.067	0.110	0.140
	Defoliation %					
0.017	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 10	32 $\pm$ 10	23 $\pm$ 10
0.034	0 $\pm$ 0	0 $\pm$ 14	0 $\pm$ 0	0 $\pm$ 0	40 $\pm$ 12	45 $\pm$ 13
0.067	0 $\pm$ 0	70 $\pm$ 3	55 $\pm$ 10	63 $\pm$ 4	85 $\pm$ 8	93 $\pm$ 14

These results indicate that ethephon is the inducer of the defoliation process, while cyclanilide is the enhancer of ethephon activity. Because cyclanilide alone did not induce defoliation but ethephon did, ethephon (ethylene) is required to trigger abscission/defoliation in bean plants. These results support the current models of hormone balance modulation of abscission (Taylor and Whitelaw, 2001; Roberts et al., 2002). The data presented herein demonstrate that cyclanilide is a potent modulator of the abscission process.

### Temperature Studies

Whole plant experiments were also conducted to determine if the cyclanilide treatment affected ethephon-induced defoliation at varying temperatures. Combining 0.067 kg a.i./ha cyclanilide with an equal rate of ethephon consistently induced  $\approx$ 75 to 85% defoliation, 5 DAT at all tested temperatures except at 16/14°C (Fig. 4). In contrast, ethephon alone was more temperature sensitive. At the highest temperature tested (30/26°C), ethephon alone induced only 23% defoliation 5 DAT, and did not induce any defoliation at other temperatures. At the lowest temperature (16/14°C), combining cyclanilide with ethephon did not induce any defoliation 5 DAT; however, 7 DAT, this treatment induced 26% defoliation. These results demonstrated that cyclanilide combined with ethephon enhanced defoliation at all temperatures.

### Auxin Transport Inhibitor Studies

Similar to cyclanilide, NPA and TIBA also enhanced defoliation when combined with ethephon (Fig. 5). As shown earlier, ethephon alone at 0.067 kg a.i./ha did not induce any defoliation 5 DAT. However, combining this rate of ethephon with 0.140 kg a.i./ha NPA or TIBA resulted in 50 and 52% defoliation, respectively. Although cyclanilide enhances defoliation more than NPA and TIBA, these results demonstrate that auxin transport inhibitors can also enhance defoliation when combined with ethephon. These results suggest that cyclanilide may be acting as an auxin antagonist or transport inhibitor, thereby enhancing ethephon efficacy.

### DISCUSSION

Whole plant experiments were conducted to determine the effect that cyclanilide has on ethephon-induced



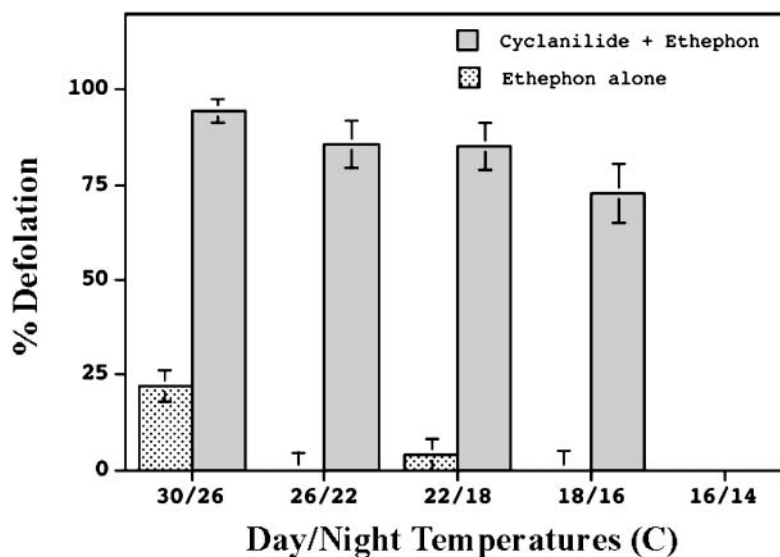


Fig. 4. Effect of decreasing day and night temperatures on red kidney bean defoliation (5 DAT) after treatment with ethephon alone at 0.067 kg a.i./ha, or in combination with 0.067 kg a.i./ha cyclanilide. Each point represents mean  $\pm$  SE,  $n = 6$ .

leaf abscission. The results demonstrated that combining cyclanilide with ethephon induced greater and more rapid defoliation compared with the application of ethephon alone, across a range of temperatures. Synergism is generally defined as a cooperative action between chemicals resulting in greater efficacy when combined than if the chemicals were applied by themselves (Akobundu et al., 1975). Cyclanilide will enhance defoliation even with low rates of ethephon. For example, while 0.067 kg a.i./ha ethephon does not induce defoliation, combining this rate of ethephon with an equal or lower rate of cyclanilide enhances defoliation. Cyclanilide alone does not induce defoliation, one manifestation of senescence, but instead will induce lateral branching (Elfving and Visser, 2005). Therefore, while ethephon is the primary inducer of the defoliation process, cyclanilide enhances ethephon activ-

ity. These results demonstrate that cyclanilide is an ethephon synergist.

According to the model for leaf abscission, inhibition of auxin transport through the petiole, or inhibition of auxin signaling in the abscission zone, should enhance ethylene-induced abscission (Taylor and Whitelaw, 2001; Roberts et al., 2002). Our results support this model because the auxin transport inhibitors NPA and TIBA were shown to enhance abscission when combined with ethephon. Similar studies using cotton whole plants or cotton explants have also shown that auxin transport inhibitors increase leaf abscission when combined with ethylene (Morgan and Durham, 1972).

Apical dominance is associated with high auxin concentrations in the growing apical meristem (Romano et al., 1993). Polar auxin transport from the apex to the roots of the plant has been linked to the polar development, differentiation, and growth of plants. Plants that are deprived of an apical auxin source either by decapitation of the apical meristem or by application of auxin transport inhibitors will induce lateral bud growth (Tamas, 1995). Therefore, it is not surprising that the application of auxin transport inhibitors such as TIBA and NPA should enhance lateral shoot growth and inhibit apical dominance. Because of the similarities in effects, cyclanilide may also act as an auxin transport inhibitor. Alternatively, our results suggest that cyclanilide synergism of ethephon activity may be due to altered auxin sensitivity and/or signal transduction.

Cyclanilide was a relatively greater synergist of ethephon-induced defoliation than were TIBA or NPA (Fig. 5). This suggests that cyclanilide may have a higher affinity to a common target site. Recent research has provided insight into the mechanism of auxin transport, and the mechanism of NPA and TIBA inhibition. Three principle gene groups have been identified as being involved in auxin transport: AUX1 genes and proteins are involved in auxin influx; and both PIN (Friml and Palme, 2002; Friml, 2003) and GNOM (Geldner et al.,

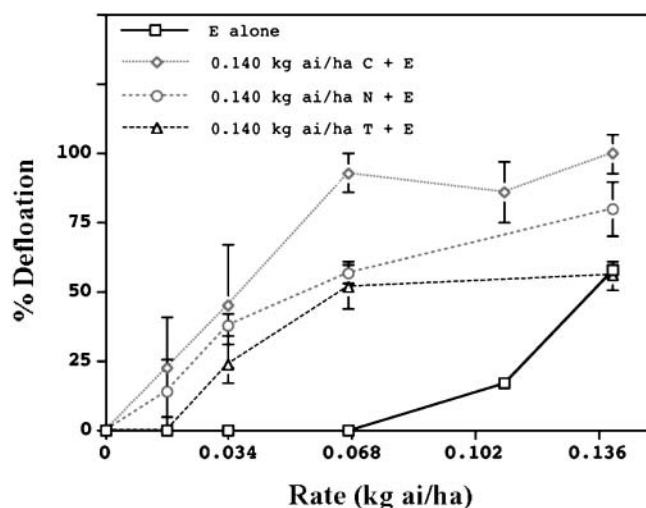


Fig. 5. Effect of cyclanilide and auxin transport inhibitors on ethephon-induced defoliation of red kidney bean, 5 DAT. Defoliation of plants sprayed with increasing rates of ethephon (E) alone, or in combination with 0.140 kg a.i./ha NPA (N), TIBA (T), and cyclanilide (C). Each point represents mean  $\pm$  SE,  $n = 3$ .

2003) genes are involved in auxin efflux (Muday et al., 2003). A fourth group involved in auxin transport, including AtMDR1 and AtPGP1, are related to the multidrug resistance (MDR) genes of animals, however their role in auxin transport is not clearly defined (Noh et al., 2001). Though all of these proteins play critical roles, the asymmetric localization of PIN proteins is necessary for the polar or directional flux of auxin. The PIN proteins rapidly cycle along actin filaments between the endosome and the plasma membrane, directing the movement of indole-3-acetic acid (IAA; Muday et al., 2003). Several proteins have been identified that bind NPA, including a high affinity NPA binding protein, which was initially thought to be an accessory protein to the auxin transporter, acting to regulate IAA efflux (Lomax et al., 1995). Different low-affinity NPA binding proteins have been identified, including the MDR-like proteins AtMDR1 and AtPGP1 (Noh et al., 2001) and plasma membrane associated aminopeptidases (Noh et al., 2003). Specifically which NPA-protein interaction accounts for the inhibition of auxin efflux remains unclear. In *Arabidopsis* spp., NPA (Geldner et al., 2001) and TIBA (Friml et al., 2002) inhibited the PIN1 cycling between the plasma membrane and the endosome. However, no inhibition of PIN1 cycling by NPA was observed in tobacco (*Nicotiana tabacum* L.) cell suspension cultures, even though NPA inhibited auxin efflux (Petrasek et al., 2003). The IAA transport polarity model described by Muday et al. (2003) suggested that NPA binding and inhibition of IAA efflux prevents the movement of PIN from the plasma membrane to the endosome, but no mechanistic explanation exists at this time. Given the similarities between the activity of cyclanilide and the auxin transport inhibitors NPA and TIBA, it is possible that cyclanilide may inhibit IAA efflux and PIN cycling within the cell. Alternatively, because of the differences in chemical structures, cyclanilide may act via a mechanism distinct from NPA and TIBA.

It is possible that cyclanilide induces greater defoliation than TIBA and NPA when combined with ethephon because of greater uptake or movement after application. Chemical formulation is also important because it affects uptake and translocation and thereby influences the performance (Beaudry and Kays, 1988). Alternatively, the structural characteristics of cyclanilide may allow for relatively greater absorption or movement to the petiole or abscission zone.

In summary, cyclanilide, NPA, and TIBA had synergistic effects on dark red kidney bean defoliation when combined with ethephon. The interaction between ethylene and auxin signaling during the abscission process is still not understood. Ethylene itself is also known to inhibit auxin transport (Beyer and Morgan, 1971; Beyer, 1973; Suttle, 1988). This research presents strong indirect evidence that auxin transport inhibition, or inhibition of the auxin signaling process, is important in enhancing leaf abscission. Our results support the model that ethylene released by ethephon induces abscission, and suggest that auxin transport inhibition will enhance ethylene sensitivity and increase leaf

abscission. Although our results suggest that cyclanilide has a similar mode of action as NPA and TIBA in promoting abscission, further investigation is necessary to determine the characteristics of cyclanilide effect on auxin signaling.

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